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Sucrose ingestion decreases seizure onset time in female rats treated with lithium and pilocarpine

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Abstract

To extend previous work concerning diet and overt seizures in rats, we tested the hypothesis that ingestion of 10% sucrose–water could reduce seizure onset time (SOT) in rats given lithium and pilocarpine. We found that female but not male rats given free access to a 10% sucrose–water solution for 3 weeks exhibited shorter SOTs than age- and sex-matched control subjects. A separate experiment determined that SOT was significantly reduced whether female rats were provided 1, 2, 3, or 4 weeks of free access to sucrose. Moreover, the daily volume of sucrose ingested was significantly correlated ($r = -0.42$) with SOT regardless of the duration of sucrose treatment (in weeks). These findings suggest that a diet supplemented with sugar can facilitate the emergence of behavioral seizures in female rats given lithium and pilocarpine. We discuss the potential role of dopamine in mediating the sucrose-induced changes in SOT.
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Keywords: Seizure onset time; Sucrose; Lithium–pilocarpine; Seizure; Rat; Dopamine

1. Introduction

Diet modifications for persons with epilepsy have focused on the management of seizures through reduction of caloric intake [1] and adjustment of the proportions of macronutrients consumed (i.e., fat, carbohydrates, proteins) [2]. Similar results have been obtained in experimental settings. Using the lithium–pilocarpine model of epilepsy, Persinger et al. [3] found that while acute (24-hour) food deprivation in rats could reduce seizure onset times (SOTs), chronic food restriction (7–14 days) produced comparable delayed SOTs following pilocarpine administration.

In a conceptually similar report, Schwechter et al. [4] revealed a weak correlation between blood glucose and seizure susceptibility in the rat following streptozocin-induced diabetic hyperglycemia. When provided with the option of consuming sugar solutions (i.e., sucrose or glucose), in addition to a nutritionally complete rodent diet, rats frequently consume about 20% more calories, wherein about half of the total daily caloric intake is derived from the sugar solution [5]. Although no changes in serum glucose were reported in rats following 4 weeks of a high-sucrose diet [6], considerable metabolic changes may have occurred that affected brain function.

In the present study, we tested the hypothesis that free access to a 10% sucrose–water solution, from which rats readily consumed 150 to 200 ml per day [7], would enhance the epileptiform activity of lithium and pilocarpine as manifested by a shorter latency between systemic injection of pilocarpine and occurrence of conspicuous rearing, rapid forelimb clonus, and falling (stage V motor seizures, see Racine [8]).

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2. Method

2.1. Subjects

A total of 69 male and female Wistar rats, approximately 90 days of age, were used in this study. All subjects were housed singly in standard metal grid cages in temperature-controlled rooms (~20 °C) for the duration of the study. Food and water were always available. The light/dark cycle was 12-hour light/12-hour darkness, with photophase onset at 0730 hours. The procedures used in this study were approved by the local animal care committee and were in compliance with the Canadian Council for Animal Care (CCAC) guidelines.

2.2. Experiment 1

To determine the effect of free sucrose consumption on SOT, 40 male and female rats were separately divided into two groups. One group of subjects received a 10% sucrose–water solution for a 3-week period; the second group received only tap water for the same duration and served as a control group. Volumes of both solutions were measured daily during the late photophase, recorded to the nearest milliliter, and refreshed for all groups. At the end of the 21-day period, rats were injected subcutaneously with lithium chloride (3 mEq/kg), followed 4 hours later by pilocarpine (30 mg/kg), to evoke overt seizures. The latency to seizure onset or seizure onset time (SOT), defined by the occurrence of forelimb clonus, rearing, and falling [8], was recorded to the nearest second for each animal.

2.3. Experiment 2

To examine whether the duration of access to sucrose influenced SOT, female rats were separated into five groups. Rats (six animals/group) were given free access to a 10% sucrose–water solution for either 1, 2, 3, or 4 weeks; the fifth group never received sucrose, and served as a control. All solutions were measured and refreshed daily. Because changing geomagnetic conditions have been reported to influence SOT [9], all groups received lithium (3 mEq/kg) and pilocarpine (30 mg/kg) on the same experimental day to minimize variability. Seizure onset time was recorded as outlined above.

3. Results

3.1. Experiment 1

The means and SEM for SOT for male and female rats given either a sucrose solution or tap water for 3 weeks are listed in Table 1. Male rats given free access to sucrose had SOTs similar to those of control males that received only tap water. However, female rats that consumed sucrose for 3 weeks had significantly shorter SOTs compared with females that received only tap water [$F(1,25) = 8.65$, $P < 0.01$, $\eta^2 = 26\%$]. Although male rats were significantly ($P < 0.001$) heavier than females with respect to mean body weight [394.2 (37.1) g vs 256.9 (31.5) g], animals of either sex that received sucrose did not differ significantly in body weight from controls. Controlling for body weight between the sexes did not change the level of statistical significance. The average amounts of 10% sucrose consumed by male and female rats did not significantly differ ($F = 2.5$, n.s.).

Table 1

Group	SOT (min)	
	Mean	SEM
Males given water	26.73	6.17
Males given sucrose	20.63	2.92
Females given water	32.69	2.72
Females given sucrose	22.89	2.01

Mean SOT and SEM for male and female rats receiving either a 10% sucrose solution or water. Only the SOT for female rats differs significantly from that of the female controls given water.

3.2. Experiment 2

The results are illustrated in Fig. 1. A one-way analysis of variance showed a marked difference in the SOTs between the groups [$F(4,28) = 6.00$, $P < 0.05$, $\eta^2 = 50\%$]. Post hoc analysis (Student–Newman–Keuls) determined that rats given free access to sucrose for either 1, 2, 3, or 4 weeks had faster SOTs than control rats with access to tap water. The mean SOTs for all rats that received sucrose for any duration were not significantly different from each other. However, polynomial analysis indicated a significantly linear ($F = 21.07$, $P < 0.001$) association between sucrose duration (in weeks) and SOT. This linear function revealed that

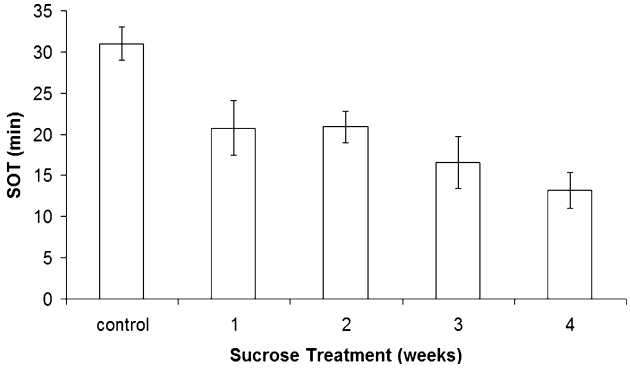

Fig. 1. Means and SEM (vertical bars) for the latency in minutes to seizure onset time (SOT) following specific durations (in weeks) of free access to 10% sucrose. All sucrose-treated female rats had significantly faster SOTs than controls.

longer periods of sucrose access were reflected by faster SOTs. The amount of sucrose consumed per 24-hour period also did not differ between the groups of rats. Because SOTs and sucrose intake did not differ between any of the sucrose treatment durations, all groups were collapsed to determine if there existed any correlation between the daily amount of sucrose consumed and the latency to seizure onset. Pearson r revealed a significant negative association ($r = -0.42$) between the mean volume of sucrose consumed per day and SOT.

4. Discussion

The results of the present study demonstrated that overt displays of limbic motor seizures in response to lithium and pilocarpine were observed earlier in female, but not male, rats provided access to a 10% sucrose solution for 3 weeks. A linear relationship between sucrose duration and SOT was discerned, and characterized by the propensity for faster SOTs in rats receiving sucrose for progressively longer durations. To the authors' knowledge, this is the first time that free access to a sucrose solution has been shown to facilitate the expression of overt seizure activity in a rat model of epilepsy.

Recent microdialysis experiments by Hajnal and Norgren [10] demonstrated that dopamine levels within the nucleus accumbens increase dramatically following sucrose feeding. Furthermore, through inhibition of the dopamine reuptake transporter (DAT) locally within the nucleus accumbens, sucrose intake could be accentuated [11]. These findings suggest that dopamine participates in the regulation of sucrose intake. More specifically, Colantuoni et al. [12] showed that after 30 days of glucose intake, dopamine D-1 receptor binding in the nucleus accumbens increased, while, conversely, D-2 receptor binding in the dorsal striatum decreased. Bello et al. [13] reported similar associations between restricted access to 0.3 M (about 10%) sucrose solution and attenuated D-2 receptor binding in the dorsolateral striatum and ventral striatum (nucleus accumbens) of rats. Together, these findings strongly suggest a prominent role for dopamine in the regulation of sucrose intake in rats.

With respect to lithium–pilocarpine-induced seizures, Barone et al. [14] demonstrated that antagonists of the dopamine D-2 receptor reduced the threshold for inducing convulsions. Furthermore, D-1 receptor stimulation has been shown to potentiate convulsions induced through systemic administration of pilocarpine [15,16]. In the light of previous work showing that sugar intake can markedly enhance the sensitivity of D-1 receptors, while conversely attenuating the activity of D-2 receptors [12,13], our observation of a potential proconvulsant effect of chronic sucrose intake on lithium–pilocarpine-induced seizures is consistent with the evidence for a role in dopaminergic transmission for mediating seizure susceptibility and limbic convulsions.

The finding that the time to display overt behavioral convulsions was significantly reduced after sucrose intake in female but not male rats is puzzling, especially in the context that both sexes consumed statistically similar volumes of sucrose. Currently, the mechanism(s) of this sex-specific effect of sucrose on SOT is not clear. One explanation for the observed effects, and extending on our working hypothesis of the role of dopamine in sucrose-induced changes in SOT, is the finding that sex differences in basal extracellular dopamine exist within the striatum of rats [17]. Interestingly, the earliest electrographic seizure activity induced by lithium and pilocarpine has been recorded in the ventral pallidum and nucleus accumbens regions [18]. These regions of the brain have been considered instrumental for the circuitry responsible for the initial facilitation and subsequent generalization of epileptic activity after lithium–pilocarpine treatment. Considering the sexual dimorphic variation in dopaminergic characteristics within this region of the brain [17], as well as the evidence of specific neurochemical alterations of dopamine after prolonged sucrose intake, we speculate that these factors may result in a preferential sensitivity of female rats to the convulsive effects of lithium and pilocarpine.

A separate experiment indicated that a significant reduction in SOT in female rats could be achieved after only 1 week of free access to sucrose. Furthermore, the magnitude of this effect on SOT remained unchanged despite an increase in the duration of sucrose access to 4 weeks. However, a noticeable linear trend toward quicker SOTs was related to the duration of sucrose access over 4 weeks. We also found that the volume of sugar–water solution consumed daily (independent of duration) was also associated ($r = -0.42$) with the latency to seizure onset. These data suggest that both volume and duration of sucrose intake can facilitate the emergence of limbic seizures following lithium and pilocarpine. However, we cannot exclude the possibility that the genotype associated with high sucrose palatability is also related to seizure susceptibility.

Dietary alterations such as fasting and ketone body production through calorie restriction have been considered effective for the management of some seizures [19]. Mantis et al. [20] hypothesized that the anticonvulsant properties of these dietary alterations are the result of a shift in brain metabolism from predominantly glucose to ketogenic substrates for energy. We suspect that the inverse may be true. Sucrose consumption in rats is associated with an approximately 20% increase in caloric intake of which up to 50% of the total daily calories are derived from sucrose [5]. This may contribute to abnormal glucose and ketone body metabolism in the brain and promote seizure-like activity.

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